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Genetic Variability in Decapod Crustacea

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Summary

Enzyme polymorphisms of 36 decapod crustacean species were examined using starch gel electrophoresis. Among 17 enzymes, variations were usually observed in αGPD, AAT, GPI, MDH, PGM and 6PGD. But one-sided variations or polymorphisms in GPI, MDH, MPI and PGM loci were found between different groups when they were taxonomically segregated (Natantia and Reptantia) or, classified according to breeding system (large female group and large male group). Observed and expected mean heterozygosities (Ho and He) were 0.042 and 0.043, respectively, and proportion of polymorphic loci (P) was 0.118 based on 32 species.

The egg sizes, hypothesized as an index for survival of lineage of a family or the parents were positively correlated with the heterozygosity within and between species. These results suggest that the maintenance of enzyme variability is strongly related to the egg size in relation to the effective population size.

Since Lewontin and Hubby (1), a great deal of data has been accumulated on gene-enzyme polymorphisms through a range of organisms including aquatic species. Selander and Kaufman (2) pointed out major taxonomic differences in electrophoretically detectable genetic variation between invertebrates ($\bar{H}$eterozygosity$=0.15$) and vertebrates ($\bar{H}=0.06$), and proposed that large mobile animals (vertebrates) face the environment as fine grained while small, less mobile animals (invertebrates) encounter it as coarse grained. A number of alternative hypothesises to interprete the correlation between the genetic variation and environmental heterogeneity have been proposed (3-10). They do not strongly consider effective population size as the basis of the selectively neutral allele hypothesis proposed by Kimura and Ohta (11). The lower enzyme heterozygosity found in *Hymenoptera* within insects (12), however, might be accepted as an example in which the social population structure and unique breeding system contribute to a decrease in the effective population size. Furthermore, Chow and Fujio (14, 15) suggested that differentiated breeding behavior between shrimp genera *Palaemon* and *Macrobrachium* within the family Palaemonidae might have caused higher genetic variability for the former and lower for the

TABLE 1. *Species of Decapod Crustacea and the Collecting Localities*

Species	Lot	Size*	Locality
uborder Natantia			
Family Penaeidae			
Metapenaeopsis dallei	2	125	Miyagi
Trachypenaeus curvirostris	3	114	Miyagi
Penaeus japonicus	3	100	Niigata, Ohita, Taiwan
Family Atyidae			
Paratya compressa improvisa	7	401	Miyagi
Family Crangonidae			
Crangon affinis	1	80	Miyagi
C. dallei	1	110	Miyagi
Family Hippolytidae			
Heptacarpus rectirostris	1	60	Miyagi
Family Palaemonidae			
Palaemon paucidens (A type)	12	761	Miyagi, Tokyo, Ishikawa, Shiga, Hiroshima
P. paucidens (B type)	8	673	Miyagi, Ibaragi, Chiba
P. orientis	1	18	Miyagi
P. macrodactylus	3	508	Miyagi
Macrobrachium nipponense	8	367	Miyagi, Ibaragi, Tokyo, Chiba, Kochi
M. formosense	4	116	Okinawa Islands, Ishigaki Islands
M. japonicum	4	200	Okinawa Island
M. lar	1	46	Iriomote Islands
Suborder Reptantia			
Family Palinuridae			
Panulirus japonicus	1	10	Tokyo Fish Market
Family Astacidae			
Procambarus clarkii	1	15	Miyagi
Family Pagridae			
Pagrus ochotensis	2	35	Miyagi
Family Drippidae			
Paradrippe granulata	1	38	Miyagi
Family Cancridae			
Cancer gibbonsulus	1	26	Miyagi
Family Atelecyclidae			
Erimacrus isenbeckii	6	312	Hokkaido, Iwate
Telmessus actidens	1	32	Miyagi
Family Portunidae			
Portunus trituberculatus	1	36	Kochi
P. sanguinolentus	1	41	Kochi
Charybdis japonica	1	11	Kochi
C. miles	1	61	Kochi
Family Xanthidae			
Epixanthus frontalis	1	21	Iriomote Island
Family Grapsidae			
Planes cyaneus	1	12	Miyagi
Hemigrapsus sanguineus	1	43	Miyagi
H. penicillatus	1	35	Miyagi
Pachygrapsus crassipes	1	35	Fukushima
Chasmagnatus convex	1	32	Okinawa Island
Helice tridens	1	32	Miyagi
Holometopus dehaani	1	39	Miyagi
H. hematocheir	1	11	Chiba
Eriocheir japonica	5	69	Okinawa island

*Maximum number of individuals exemined per locus.

latter. It is very difficult to estimate the actual effective size of populations, but we might be able to know the degree to which some factors influence the effective population size. This study examines the distributions of enzyme variation using 36 decapod crustacean species, and discusses some factors probably related to the effective population size and consequently affecting the level of enzyme variabilities.

Methods

36 species of decapod crustacea were collected from marine and freshwater areas and used for starch gel electrophoresis, using extracts mainly of the abdominal and pereiopod muscles and, rarely, eyes. Species and collection localities are given in Table 1. All of them, except for one species *Procambarus clarkii* which was introduced from the USA, are native species of Japan.

17 enzymes acid phosphatase (ACP), α-glycerophosphate dehydrogenase (αGPD), aspartate aminotransferase (AAT), esterase (EST), fumarase (FUM), galactose dehydrogenase (GAL), glucose phosphate isomerase (GPI), glucose-6-phosphate dehydrogenase (G6P), isocitrate dehydrogenase (IDH), lactate dehydrogenase (LDH), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), malic enzyme (ME), mannose phosphate isomerase (MPI), phosphoglucomutase (PGM), 6-phosphogluconate dehydrogenase (6PGD), and sorbitol dehydrogenase (SDH) were examined. Procedures for gel electrophoresis follow Chow and Fujio (13). Staining recipes are based on Shaw and Prasad (16) except for AAT and MPI which were from Sugita and Fujio (17) and Dando (18), respectively.

Results

1. Genetic Interpretation of Electrophoretic Patterns

Enzyme variabilities of 36 species are given in TABLE 2. A locus was defined as polymorphic when the frequency of the most common allele was less than 0.95.

αGPD phenotypes were always single or three banded indicating the dimeric structure of this enzyme. All individuals examined in 8 palaemonid shrimps and *Procambarus clarkii* represented three banded phenotype assumed to be coded by two loci, each of which is producing single polypeptide with heterodimer between them. Variations were observed in 5 species. Likewise AAT was interpreted as a dimeric structure, but formation of heterodimer between the isozymes when they are obviously produced by two loci, was not observed. ACP was examined in 33 species but scorable zymograms were obtained in 7 of 18 species in which the activities were found. Variation was observed in *Helice tridens*, indicating the dimeric structure. EST appeared as multi-banded or zone phenotypes. Although all of the species examined showed activity, 18 species provided scorable zone as loci. When the scorable variations were observed, this enzyme was assumed as a monomeric structure. Among 17 species in which FUM activity was

TABLE 2. *Summary of the Enzyme Variabilities*

Species	ACP	AAT	αGPD	EST#	FUM	GAL
Metapenaeopsis dallei	+	P*	P*	+	na	−
Trachypenaeus curvirostris	M	na	M	+	na	−
Peenaeus japonicus	2M	M	M	+	2M	−
Paratya compressa improvisa	−	2M	+	P+M	−	−
Crangon affinis	+	na	+	+	2M	−
C. dallei	an	na	+	+	2M	−
Heptacarpus rectirostris	M	−	−	−	−	−
Palaemon paucidens (A type)	+	P*+M	2M	M	+	na
P. paucidens (B type)	+	2M	2M	M	+	na
P. orientis	+	2M	2M	M	+	na
P. macrodactylus	+	2M	2M	M	+	−
Macrobrachium nipponense	+	P*+M	2M	M	+	−
M. formosense	+	P*+M	2M	M	+	−
M. japonicum	+	2M	2M	M	+	−
M. lar	+	2M	2M	M	+	−
Panulirus japonicus	na	−	M	+	−	−
Procambarus clarkii	na	M	2M	+	na	−
Pagrus ochotensis	M	−	na	+	2M	−
Paradrippe granulata	−	−	−	−	−	−
Cancer gibbonsulus	na	2M	na	+	−	−
Erimacrus isenbeckii	na	P+M	P*	M	−	M
Telmessus actidens	na	2M	M	M	na	−
Portunus trituberculatus	na	P	M	2M+P	−	−
P. sanguinolentus	na	M	P	2M	−	−
Charybdis japonica	M	M	M	+	−	−
C. miles	nb	M	P	+	−	−
Epixanthus frontalis	na	2M	na	+	−	−
Planes cyaneus	−	2P	−	−	−	−
Hemigrapsus sanguineus	M	++P*	na	P	−	−
H. penicillatus	+	M+P*	M	2M	−	−
Pachygrapsus crassipes	na	M+P*	M	+	−	−
Chasmagnatus convex	na	P*+M	P*	+	−	−
Helice tridens	P	2P*	M	P+M	−	−
Holometopus dehaani	na	2M	M	M	M	−
H. hematocheir	na	2M	M	+	−	−
Eriocheir japonica	na	M+P*	+	4M	−	−
Total number of species	7	29	26		5	1

EST was not included for comparisons of enzyme variation because of the indistinct variation. P: Polymorphic (frequency of the most common allele is not greater than 0.95), P*: variation, M: No variation, +: Activity, na: No activity, and −: Not examined.

of 36 Decapod Crustacean Species

Enzymes										
GPI	G6P	IDH	LAP	LDH	MDH	ME	MPI	PGM	6PGD	SDH
P*	–	M	na	2M	P+M	M	P	2P	P	–
P	–	M	na	M	M+P*	2M	P*	P*+P	P*	–
M+P*	–	M	na	2M	P*+M	2M	P	2P*	P	–
P	–	M	–	M	+	2M	P*	P*	M	–
P*	–	2M	–	2M	M+P*	2M	P*	P*	M	–
P	–	2M	na	2M	M+P*	2M	P	P	M	–
P*	–	–	–	M	P+P*	M	P	M	–	–
P	–	M	na	M	P*+P	2M	P	P	P	na
P*	–	M	na	M	P+M	2M	P	P	P*	na
P*	–	M	na	P	2M	2M	P	M	M	na
P	–	M	na	M	2P*	2M	P	P	M	na
P*	–	M	na	M	P*+M	2M	P	P	P*	na
P*	–	M	na	M	P*+M	2M	P	P	M	na
P	–	M	na	M	2P*	2M	M	P*	M	na
P*	–	M	na	M	2M	2M	P*	P*	M	na
P	–	+	2M	4M	2M	M	M	M	+	–
2M	na	M	P	M	2M	2M	M	M	M	–
P	–	2M	–	P*+M	2M	M	–	P*	P+2M	–
P	–	–	–	M	–	P	–	–	–	–
P	–	M	na	M	M+P*	+	M	P*	P*	na
M	na	M	na	2M	2M	2M	M	M	P*	M
M	–	M	M	M	2M	2M	M	M	2M	–
M	na	2M	na	M	2M	2M	M	M	M	–
P*	na	2M	na	M	M+P*	2M	M	M	M	–
P	na	2M	M	M	2M	2M	M	P*	M	–
P*	na	2M	M	2M	2P*	2M	M	M	M	–
P	na	M	na	M	2M	+	P	M	P	–
M	–	M	–	M	–	–	–	–	M	–
P*	na	M	M	M	2M	2M	2M	M	P*	–
P	M	M	M	M	M+P*	+	2M	P*	M	–
P*	na	M	M	M	2M	+	2M	P	M	–
P	na	M	M	M	M+P*	2M	2M	M	M	–
M	na	P	M	M	2M	+	2M	M	M	–
P	na	M	M	M	2M	+	2M	M	M	–
M	na	M	M	M	2M	+	2M	M	M	–
P	na	M	M	M	2M	2M	2M	P	P*	–
36	1	33	13	36	33	28	33	34	33	1

found, only 5 species provided scorable zymograms. Since none of the species showed variation, tetrameric structure suggested in human FUM (19) could not be confirmed. GAL was scorable in *Erimacrus isenbeckii* with no variation. GPI was clearly a dimeric enzyme. 2 loci were found in *Penaeus japonicus* and *Procambarus clarkii*, and the other 34 species had one locus. Variations were observed in 29 species in which polymorphisms were found in 16 species. G6P activity appeared only in *Hemigrapsus penicillatus* among 15 species examined, with no variation. *Helice tridens* was the only one species showing variation in IDH. Single and three banded phenotypes clearly indicated a dimeric structure for this enzyme. 2 *Crangon* spp., *Pagrus ochotensis* and 4 portunid crabs showed 2 banded phenotypes assumed as products of two loci without heterodimer between them. LDH phenotypes were scorable for 36 species. Variations were observed in *Palaemon orientis* and *Pagrus ochotensis* with single and quadruple banded phenotypes. *Metapenaeus dallei*, *Penaeus japonicus*, 2 *Crangon* spp., and *Pagrus ochotensis* had 2 loci without heterotetramer, while formation of heterotetramer between the 2 loci of *Erimacrus isenbeckii* and *Charybdis miles* was observed. Extracts from abdominal and pereiopod muscle produced different LDH isozymes in *Panulirus japonicus*. One fast zone was obtained from pereiopod muscle, and two slow thin and thick zones were observed for abdominal muscle. Furthermore, the slowest thick zone for abdominal muscle was segregated into two thin bands in eye extracts. As a results, 4 tissue specific LDH loci were confirmed in this species. LDH activity in *E. isenbeckii* indicated existence of different isozymes even between portions of pereiopod. Muscle of cheliped provided one fast band, while that of walking leg showed the same fast and slower band with a heterotetramer between them. LAP activity appeared as single or two bands indicating one or two loci. Variation was observed in *Procambarus clarkii*, and the phenotypes indicated that this enzyme is a monomeric structure. MDH activity appeared in 2 zones for 33 species. Heterozygotes were three banded in each zone in agreement with a dimeric structure. Variations were observed in 17 species. ME phenotypes were scorable for 28 out of 35 species examined, appearing as single or two bands indicating one or two loci. Variation was found *Paradrippe granulata* as a thin banded homozygote and thick banded heterozygote, but the tetrameric structure observed in some animals (20) was not confirmed here. Variations in MPI were found in 15 out of 33 species examined, and obviously indicated a monomeric structure. All of the individuals examined in 8 grapsid crabs showed 2 banded phenotypes indicating 2 loci. Parallel results were obtained in PGM. Only 3 penaeid shrimps were assumed to have 2 loci, and 2, 3 and 4 banded phenotypes were observed in these species. 6PGD was apparently a dimeric structure. Variations were observed in 12 species. SDH activity was found in *Erimacrus isenbeckii* among 10 species examined, with no variation.

TABLE 3. *Genetic Variations in 32 Species of Decapod Crustacea*

Species	No. of loci	Ho	He	P	D
Metapenaeopsis dallei	13	0.120	0.119	0.347	0.008
Trachypenaeus curvirostris	13	0.070	0.062	0.180	0.129
Penaeus japonicus	19	0.045	0.044	0.105	0.023
Paratya compressa improvisa	12	0.057	0.055	0.155	0.036
Crangon affinis	14	0.010	0.010	0.000	0.000
C. dallei	14	0.050	0.063	0.214	−0.206
Palaemon paucidens (A type)	15	0.097	0.100	0.261	−0.030
P. paucidens (B type)	15	0.078	0.081	0.217	−0.037
P. orientis	15	0.048	0.056	0.133	−0.143
P. macrodactylus	15	0.079	0.080	0.200	−0.013
Macrobrachium nipponense	15	0.043	0.043	0.150	0.000
M. formosense	15	0.055	0.062	0.133	−0.113
M. japonicum	15	0.036	0.036	0.084	0.000
M. lar	15	0.012	0.011	0.000	0.091
Procmbarus clarkii	15	0.022	0.028	0.067	−0.214
Pagrus ochotensis	15	0.075	0.058	0.133	0.293
Cancer gibbonsulus	10	0.036	0.034	0.100	0.059
Erimacrus isenbeckii	17	0.024	0.026	0.059	−0.077
Telmessus actidens	16	0.000	0.000	0.000	0.000
Portunus trituberculatus	16	0.043	0.060	0.125	−0.283
P. sanguinolentus	15	0.045	0.043	0.067	0.047
Charybdis japonica	15	0.018	0.017	0.067	0.059
C. miles	16	0.016	0.015	0.059	0.067
Epixanthus frontalis	10	0.062	0.061	0.300	0.016
Hemigrapsus sanguineus	16	0.037	0.030	0.063	0.233
H. penicillatus	16	0.019	0.018	0.063	0.056
Pachygrapsus crassipes	13	0.024	0.029	0.077	−0.172
Chasmagnatus convex	15	0.019	0.018	0.067	0.056
Helice tridens	17	0.033	0.033	0.176	0.000
Holometopus dehaani	16	0.030	0.031	0.063	−0.032
H. hematocheir	13	0.000	0.000	0.000	0.000
Eriocheir japonica	18	0.034	0.043	0.100	−0.209
Mean		0.042	0.043	0.118	−0.011
±SD		0.028	0.028	0.086	0.123

2. *Genetic Variation in Each Species*

It is important that we have compared variations using almost the same enzymes in each species. Two estimates of genetic variability, the mean heterozygosities (Ho and He) and the proportion of polymorphic loci (P) in 32 species were calculated, and each variability was averaged by the number of lots

examined. The results are given in TABLE 3. Observed mean heterozygosity (Ho) ranged from 0.000 to 0.120 with a mean of 0.042 ± 0.028, expected heterozygosity (He) from 0.000 to 0.119 with a mean of 0.043 ± 0.028, and proportion of polymorphic loci (P) from 0.000 to 0.347 with a mean of 0.118 ± 0.086. The index, $D = (Ho-He)/He$, was used to evaluate heterozygote excess or deficiency for each species. The D value ranged from -0.283 to 0.293 with a mean of -0.011 ± 0.123. Generally speaking, there was no notable difference between Ho and He through all the species. In almost all species, no significant departures of observed phenotypes from Hardy-Weinberg's expected number of phenotypes at each locus were found. Close agreement with the H-W equilibrium supports propriety of genetic interpretations of electrophoretic patterns very well.

3. *Comparisons of Genetic Variabilities between Different Groups*

Species are grouped by 3 categories. The first category is taxonomic separating two major suborders, Natantia and Reptantia. The second is related to breeding system (14), by which one group whose females tend to be bigger than males (large female group) and another whose males are usually bigger than females (large male group) were separated. All of the Reptantia and *Macrobrachium* spp. of Natantia belonged to the large male group, and other Natantian species were grouped into the large female group. The third is based on whether the species is substantially marine or not. We expect that the environmental conditions for the life cycle of non-substantially marine species change more drastically than those of substantially marine species. Substantially marine species are as follows; 3 penaeids, 2 *Crangon* spp., *Heptacarpus rectirostris*, *Palaemon orientis*, *P. macrodactylus*, *Panulirus japonicus*, *Pagrus ochotensis*, *Paradrippe granulata*, *Cancer gibbonsulus*, *Erimacrus isenbeckii*, *Telmessus*

TABLE 4. *Comparisons of Genetic Variabilities between Different Groups*

Category I		Ho	He	P
Natantia	(14)*	0.057 ± 0.030^a	0.058 ± 0.031^b	0.154 ± 0.094^c
Reptantia	(18)	0.030 ± 0.019^a	0.030 ± 0.018^b	0.088 ± 0.067^c
Category II		Ho	He	P
Large ♀	(10)	0.065 ± 0.031^d	0.067 ± 0.030^e	0.181 ± 0.093^f
Large ♂	(22)	0.031 ± 0.019^d	0.032 ± 0.018^e	0.089 ± 0.066^f
Category III		Ho	He	P
Marine	(20)	0.043 ± 0.028^g	0.043 ± 0.028^h	0.123 ± 0.091^i
Others	(12)	0.040 ± 0.028^g	0.042 ± 0.029^h	0.108 ± 0.079^i

* Number of species. a, b, c, d, e and f are significantly different. None of g, h and i are significantly different.

actidens, 4 portunids, *Epixanthus frontalis*, *Planes cyaneus*, 2 *Hemigrapsus* spp., *Pachygrapsus crassipes*, and *Helice tridens*.

These results are summarized in TABLE 4. Significant differences between pairwise comparisons of all variabilities in categories I and II were found, but not in category III. This indicates that Natantia or large female groups have higher variabilities than Reptantia or large male groups. To compare the genetic variabilities between different groups at each enzyme, we concentrated on 7 enzymes (αGPD, AAT, GPI, MDH, MPI, PGM and 6PGD) in which genetic variations were usually observed. For each category, the number of species showing no variation (M), variation (P*) or polymorphism (P) was counted. The results are summarized in TABLE 5, and a test of significance between the groups was carried out by 2×2 test of independence using G-statistics (21). No significant differences were observed between marine and others groups at any enzyme but for αGPD, while significant differences were found between Natantia and Reptantia or large female and large male groups at GPI, MDH, MPI and PGM. These differences are due to the one-sided variations or polymorphisms in each enzyme between the groups, and indicated that the variabilities in each enzyme of Natantia are above those of Reptantia, also variabilities in each enzyme of the large female group were above those of the large male group.

4. Correlation between Heterozygosity and Egg Size

Using egg size as an index for effective population size, we examined the correlation between heterozygosity and egg size. Egg volume was calculated according to Chow and Fujio (13). Expected mean heterozygosity (He) and egg volume (mm^3)of 17 species and 14 local populations of *Palaemon paucidens* which is known by the great variation in the egg size (22), are given in TABLE 6. Sperman rank correlation coefficient (r_s) was calculated for 14 samples within *P. paucidens*, for 17 species excluding *P. paucidens*, and for all 31 samples. Significant positive correlations were obtained for *P. paucidens* ($r_s=0.707$, $P<0.005$) and for all 31 samples ($r_s=0.815$, $P<0.005$). No significant correlation was observed for 17 species excluding *P. paucidens*, but still highly positive ($r_s=0.360$, $0.1<P<0.25$). These results suggest that heterozygosity is positively correlated with egg size within and between species.

Discussion

We could not or did not find any environmental factor which might influence the level of enzyme variations except for a rough criterion of classifying substantially marine species from others (category III), for the species grouping. Most of the species were collected from a temperate zone and shallow water, and no available data could be obtained on whether the environment that the species experiences were homogeneous or heterogeneous through space or time.

TABLE 5. *Comparisons of Number of Species Showing Different Variabilities between Different Groups*

Enzyme	Category	P+P*	M	G	P	P*+M	G
αGPD	I Natantia	1	10	1.568	0	11	2.456
	I Reptantia	4	10		2	12	
	II Large ♀	1	6	0.210	0	7	1.380
	II Large ♂	4	14		2	16	
	III Marine	4	11	4.586*1	2	13	0.066
	III Others	0	10		1	9	
AAT	I Natantia	4	7	0.518	0	11	3.07
	I Reptantia	9	9		3	15	
	II Large ♀	2	5	1.017	0	7	1.765
	II Large ♂	11	11		3	19	
	III Marine	8	9	0.084	3	14	3.447
	III Others	5	7		0	12	
GPI	I Natantia	15	0	8.734*3	6	9	0.206
	I Reptantia	14	7		10	11	
	II Large ♀	11	0	5.819*3	5	6	0.005
	II Large ♂	18	7		11	14	
	III Marine	19	5	0.091	10	14	0.225
	III Others	10	2		6	6	
MDH	I Natantia	12	2	12.333*3	4	10	7.624*2
	I Reptantia	5	14		0	19	
	II Large ♀	9	1	9.496*3	4	6	10.916*3
	II Large ♂	8	15		0	23	
	III Marine	11	11	0.060	2	20	0.540
	III Others	6	5		2	9	
MPI	I Natantia	14	1	30.403*3	10	5	15.190*3
	I Reptantia	1	17		1	17	
	II Large ♀	11	0	24.614*3	8	3	11.594*3
	II Large ♂	4	18		3	19	
	III Marine	9	12	0.160	7	14	0.000
	III Others	6	6		4	8	
PGM	I Natantia	13	2	11.183*3	7	8	5.784*1
	I Reptantia	6	13		2	17	
	II Large ♀	9	2	4.741*1	6	5	4.783*1
	II Large ♂	10	13		4	19	
	III Marine	11	11	0.887	5	17	1.311
	III Others	8	4		5	7	
6PGD	I Natantia	6	8	0.441	3	11	0.737
	I Reptantia	6	13		2	17	
	II Large ♀	5	5	1.132	3	7	2.264
	II Large ♂	7	16		2	21	
	III Marine	8	13	0.076	4	17	0.738
	III Others	4	8		1	11	

*1 $0.01<P<0.05$, *2 $0.005<P<0.01$, *3 $P<0.005$.

Somero and Soule (6) examined genetic variation in teleost fishes from different thermal regions, and suggested that polymorphism patterns can be better explained by a time-divergent model of selection than by the nitche-variation hypothesis. Significant one-sided enzyme variabilities and heterozygosity differences between Natantia and Reptantia (category I) might have been contributed to the differential appearance time between the taxa. But fossil records indicate that the appearance of origins of penaeids in Natantia and Reptantia preceded caridea which occupies a large part of Natantia, and that Natantia and Reptantia were segregated by means of the adaptations and habits, not the origin (23). Furthermore, it is unlikely that most of Reptantia recently experienced more severe bottlenecking than Natantia. These suggest that the difference in genetic variation between the taxa is not due to the time-divergent phylogenetical differences. Chow and Fujio (14) reported significant difference between the average heterozygosities of two closely related genera, *Palaemon* and *Macrobrachium*, and suggested that the differences were caused by the breeding system. Nevo (24) has pointed out that genetic variation is affected by breeding system in plants and animals. In this study, the above suggestion might be extended and applied to higher groups of decapod crustacean species as seen between the large female and large male groups (category II). The differences in enzyme variabilities and heterozygosities observed between the taxa might be due to the fact that most species in the large male group belong to Reptantia. It is also probable that the differences between the taxa are due to the fact that crabs occupy most of Reptantia which have small egg sizes, considering the significant positive correlation between the egg size and heterozygosiy. Although effective population size has been recognized as one of the factors influencing the level of heterozygosity (11), the effective population size in most organisms has hardly been estimated. It is not proper to extrapolate the effective size from the actual abundance without knowing the differences in breeding system. Known as K- and *r*-selections, it is believed that the animals whose eggs tend to be big necessarily produce a small number of eggs, while the animals whose eggs tend to be small produce a large number of eggs. For the former, the few individuals produced by each family of parents play an imortant role in maintaining the population or species. For the latter, on the other hand, the elmination of a family lineage or the parents is not critical ; the survivors from the huge amount of larvae are enough to maintain the population or species. We hypothesized that this essential difference has affected the maintenance of genetic variability related to effective population size. It is an interesting phenomenon that the positive correlation betweeen the egg size and heterozygosity was found among local populations of *P. paucidens*. Although absolute population size of the lakes must be greater than that of the ponds, the heterozygosity levels are higher in the ponds than in the lakes (See TABLE 6). Furthermore, the genetic heterogeneity

TABLE 6. *Heterozygosity and Egg Size in 31 Samples*

Species	Locality	He	Mean egg Volume mm³
Palaemon paucidens (A type)	Miyagi (pond)	0.111	0.998
P. paucidens (A type)	Miyagi (pond)	0.138	0.993
P. paucidens (A type)	Miyagi (pond)	0.094	0.991
P. paucidens (A type)	Miyagi (river)	0.109	0.975
P. paucidens (A type)	Miyagi (pond)	0.094	0.956
P paucidens (A type)	Tokyo (river)	0.067	0.946
P. paucidens (A type)	Miyagi (pond)	0.147	0.860
P. paucidens (A type)	Ishikawa (lake)	0.055	0.659
P. paucidens (B type)	Miyagi (river)	0.092	0.619
P. paucidens (B type)	Miyagi (river)	0 078	0.538
P. paucidens (B type)	Miyagi (river)	0.090	0.520
P. paucidens (B type)	Miyagi (river)	0.081	0.497
P. paucidens (A type)	Hiroshima (river)	0 061	0.426
P. paucidens (A type)	Shiga (lake)	0.062	0.142
Paratya compressa improvisa	Miyagi (pond)	0.047	0 134
Macrobrachium japonicum	Okinawa (river)	0.036	0.108
Palaemon macrodactylus	Miyagi (coast)	0.080	0.101
Macrobrachium nipponense	Miyagi, Tokyo (pond) Ibaragi (lake) Chiba, Kochi (river)	0.043	0.082
M. lar	Iriomote (river)	0.011	0.081
M. formosense	Okinawa (river)	0.062	0 080[1]
Eriocheir japonica	Okinawa (river)	0.043	0.048[2]
Chasmagnatus convex	Okinawa (river)	0.018	0.041
Portunus trituberculatus	Kochi (coast)	0.060	0.036
Holometopus dehaani	Miyagi (river)	0.031	0.025
Pachygrapsus crassipes	Fukushima (coast)	0 029	0.022[2]
Charybdis miles	Kochi (coast)	0.015	0 019
Holometopus hematocheir	Chiba (river)	0.000	0.019[2]
Portunus sanguinolentus	Kochi (coast)	0.043	0.013
Charybdis japonica	Kochi (coast)	0.017	0 013
Hemigrapsus sanguineus	Miyagi (coast)	0.030	0.012
Penaeus japonicus	Niigata, Ohita, Taiwan (coast)	0 044	0.007[3]

Egg volumes were calculated from egg diameters measured by 1) Shokita (25), 2) Kurata and Matsuda (26), and 3) Hudinaga (27).

among the pond populations in spite of their close proximity to one another (maximum geographic distance of 7 km) indicates that each population experiences bottlenecking and random genetic drift, not differential selective forces. Therefore, the larger egg guarantees the survival of lineage of each family or the

parents, consequently maintaining effective population size. The smaller egg size but larger number of eggs maintains the population irrespective of survival of each array, resulting in a uniform genetic structure within the population.

Our attempt to interpret the correlation between genetic variation and egg size is preliminary. The reproductive biology for each species should be clarified in detail, and other factors like clutch size, larval period and age at maturation must be studied in relation to the maintenance of effective population size.

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